J. Fac. Fish. Anim.
Husb., Hiroshima Univ. (1973), **12**:117～120

Fertility of Turkey Spermatozoa Frozen for Two Years

Moriyuki WATANABE and Yoshiteru MIYASHIMA

Department of Animal Husbandry, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama

(Tables 1-2)

Attempts to store diluted turkey semen in liquid state have been carried out by many investigators such as MORAVEC *et al.* (1954)[1], HARPER (1955)[2], CARTER *et al.* (1957)[3], WILCOX *et al.* (1960)[4], HARRIS *et al.* (1963)[5], BAJPAI *et al.* (1963)[6] and HARRIS (1968)[7], but it seems that very little research has been done until today on deep freezing preservation of turkey semen, with the exception of RAJAMANNAN (1968)[8], MACPHERSON *et al.* (1969)[9] and WATANABE *et al.* (1970, 1972)[10),11].

The present experiment was made to apply our techniques of frozen fowl semen to the artificial breeding of turkeys which seem to become important meat producing birds in the future.

MATERIALS AND METHODS

Semen ejaculates were collected from two Bronze Turkey toms of thirty months of age. The experimental semen was processed and frozen by the procedures used for fowls by WATANABE *et al.* (1970)[12]. The composition of the diluent for the frozen semen was as follows; 6.5% $C_6H_{12}O_6$ solution 85 plus fresh egg yolk 15, the turkey spermatozoa in the diluents giving a final concentration of 7% glycerol. In measurement of the depression of the freezing point of the diluent, the semi-micro-osmometer made by the Knuer Co. of West Germany was used. The freezing point depression of the buffer was −0.707°C. The collected semen was diluted to four fold with the above diluent and placed at 5°C for 5 minutes immediately after collection. Afterwards about 0.2 *mℓ* of the diluted semen were dispensed into 1 *mℓ* straw ampule and sealed. Following equilibration for 5 minutes, the samples were subjected to pre-freezing in the evaporated vapour of liquid nitrogen (about −110°C～−120°C) for 3 minutes and then stored in the liquid nitrogen. When the samples were thawed, a small drop of semen was placed between a slide and cover glass and examined under a microscope at 37°C. Motility

of samples was scored by a scale of five point (卅, 卄, +, ±, −). The fertility of the frozen semen stored for 638, 642, 643 and 739 days at −196°C by the above method was examined by artificial insemination using 6 Bronze Turkey hens, at about thirty months of age. The percent of fertility was measured as that for three weeks following insemination.

RESULTS AND DISCUSSION

Table 1 indicates the characteristics of the semen at intervals of storage up to 739 days. The mean percent of motile spermatozoa in thawed semen after storage for 638, 642, 643 and 739 days was 87.5% (over 卄), ranging from 80 to 95%; that for 638 days was 90%; that for 642 days, 80%; that for 643 days, 90% and that for 739 days 90%. Thus the influence of storage at −196°C on the motility of frozen turkey semen was not striking. The differences of the motility of spermatozoa after storage as above mentioned may be depending upon the original quality of the samples.

The freezing point depression of undiluted turkey semen in the present experiment was −0.77°C on the average and that of 6.5% glucose egg yolk diluent was −0.707°C. The values of the two solutions nearly coincide.

Table 1. The motility of the turkey spermatozoa by various storing periods

Toms No.	Storing period (days)	Motility of raw semen	Motility after thawing	Injected hens No.
23	739	95	90	21, 25
24	643	90	90	26
23	642	80	80	22
23	638	90	90	23, 24

Table 2. Fertility of the turkey spermatozoa stored for 638 to 739 days

Date / No.	First week May 24	25	26	27	28	29	30	Second week Jun. 31	1	2	3	4	5	6	Third week 7	8	9	10	11	12	13	Freezing period of the semen (days)
21	○		○	○			○		○		○	○	○		○	○		○	○			739
22	○	○				○	○	○			○		○		○	○				○	○	642
23	○						○			○		○		○		○						638
24		○	○				○							○								638
25	○		○					○	○	○		×	○		○	○		○		×	○	739
26	○			○					○		○	○		○					○			643

Note: × Fertile egg; ○ Infertile egg

The results of one single insemination of the semen samples stored for from 638 to 739 days are shown in Table 2.

The fertile eggs were not produced at all during the first week counted from the second day following insemination, but in the second and third week counted from the ninth and sixteenth day following insemination, one fertile egg was produced respectively. Therefore the fertility measured as that for three weeks following insemination was 3.8%. However, these fertile eggs died on the way of incubation.

In the present experiment, although the fetility was very low and no chick was raised, considering the facts that fertile eggs were produced by the hen through the semen sample stored for 739 days in liquid nitrogen, it must be admitted that the use of frozen turkey semen for artificial insemination offers a great possibility in the future.

SUMMARY

Turkey semen diluted with a 6.5% glucose egg yolk solution containing 7% glycerol in the final concentration and quickly frozen by liquid nitrogen was stored at −196°C for 638 to 739 days. Motility and fertility of spermatozoa in the thawed semen were studied. The following results were obtained.

1. Percentage of motile spermatozoa in the thawed semen stored for 638, 642, 643 and 739 days was on average 87.5% (over ++) ranging between 80 and 95%. Spermatozoa motility did not appear to be adversely affected by the long storage time.

2. Fertility for three weeks following insemination was 3.8%.

REFERENCES

1) Moravec, D. F., F. E. Mussehl and D. M. Pace: Poult. Sci., **33**:1126-1129, 1954.
2) Happer, J. A.: ibid., **34**:1289-1291, 1955.
3) Carter, R. D., M. G. McCartney, V. D. Chamberlin and J. W. Wyne: ibid., **36**:618-621, 1957.
4) Wilcox, F. H. and C. S. Shaffner: ibid., **39**:1580-1581, 1960.
5) Harris, G. C. Jr., T. D. Hobbs, J. E. Brown and L. B. Warren: ibid., **42**:536-538, 1963.
6) Bajpai, P. K. and K. E. Brown: ibid., **42**:888-893, 1963.
7) Harris, G. C. Jr.: ibid., **47**:397-404, 1968.
8) Rajamannan, A. H. J.: 6th Inter. Cong. Anim. Reprod. A. I., Paris, **2**:1641-1643, 1968.
9) Macpherson, J. W. S. Chatterjee and G. W. Friars: Can. J. Comp. Med., **33**:37-38, 1969.
10) Watanabe, M. and S. Kato: J. Fac. Fish. Anim. Husb. Hiroshima Univ., **9**:133-138, 1970.
11) Watanabe, M., T. Harada and S. Kato: Jap. J. Anim. Reprod., **17**:151-153, 1972.
12) Watanabe, M., M. Miura and Y. Moda: Jap. Poult. Sci., **7**:23-29, 1970.

二年間凍結した七面鳥精子の受精率について

渡辺　守之・宮島　義輝

鶏精子の長期凍結保存に引きつづき，マッサージ法によって採取した七面鳥精子をグリセリンの最終濃度7％の5.5％ブドウ糖・卵黄希釈液で4倍に希釈し，急速法によって−196°Cの液体窒素中に638日～739日間凍結保存した精子の融解後の活力および同精液使用による受精率を調べた結果は次の如く要約される。

1．液体窒素中に638日，642日，643日および739日間凍結保存した七面鳥精子の融解後の活力は80～95％，平均87.5％（＋＋以上）で，長期保存により精子活力が著しい影響を受けるとは思われない．

2．同精液使用による注入後3週間の受精率は3.8％であった．